

EXHIBIT D

(PART 2 OF 3)

US 6,565,509 B1

9

Often, the narrower the width of the sensor **42**, the less pain the patient will feel during implantation of the sensor and afterwards.

For subcutaneously implantable sensors **42** which are designed for continuous or periodic monitoring of the analyte during normal activities of the patient, a distal end **67** of the sensor **42** which is to be implanted into the patient has a width **53** of 2 mm or less, preferably 1 mm or less, and more preferably 0.5 mm or less. If the sensor **42** does not have regions of different widths, then the sensor **42** will typically have an overall width of, for example, 2 mm, 1.5 mm, 1 mm, 0.5 mm, 0.25 mm, or less. However, wider or narrower sensors may be used. In particular, wider implantable sensors may be used for insertion into veins or arteries or when the movement of the patient is limited, for example, when the patient is confined in bed or in a hospital.

Returning to FIG. 2, the proximal end **65** of the sensor **42** may have a width **55** larger than the distal end **67** to facilitate the connection between contact pads **49** of the electrodes and contacts on a control unit. The wider the sensor **42** at this point, the larger the contact pads **49** can be made. This may reduce the precision needed to properly connect the sensor **42** to contacts on the control unit (e.g., sensor control unit **44** of FIG. 1). However, the maximum width of the sensor **42** may be constrained so that the sensor **42** remains small for the convenience and comfort of the patient and/or to fit the desired size of the analyte monitor. For example, the proximal end **65** of a subcutaneously implantable sensor **42**, such as the sensor **42** illustrated in FIG. 1, may have a width **55** ranging from 0.5 mm to 15 mm, preferably from 1 mm to 10 mm, and more preferably from 3 mm to 7 mm. However, wider or narrower sensors may be used in this and other in vivo applications.

The thickness of the substrate **50** may be determined by the mechanical properties of the substrate material (e.g., the strength, modulus, and/or flexibility of the material), the desired use of the sensor **42** including stresses on the substrate **50** arising from that use, as well as the depth of any channels or indentations formed in the substrate **50**, as discussed below. Typically, the substrate **50** of a subcutaneously implantable sensor **42** for continuous or periodic monitoring of the level of an analyte while the patient engages in normal activities has a thickness of 50 to 500 μm and preferably 100 to 300 μm . However, thicker and thinner substrates **50** may be used, particularly in other types of in vivo sensors **42**.

The length of the sensor **42** may have a wide range of values depending on a variety of factors. Factors which influence the length of an implantable sensor **42** may include the depth of implantation into the patient and the ability of the patient to manipulate a small flexible sensor **42** and make connections between the sensor **42** and the sensor control unit **44**. A subcutaneously implantable sensor **42** for the analyte monitor illustrated in FIG. 1 may have a length ranging from 0.3 to 5 cm, however, longer or shorter sensors may be used. The length of the narrow portion of the sensor **42** (e.g., the portion which is subcutaneously inserted into the patient), if the sensor **42** has narrow and wide portions, is typically about 0.25 to 2 cm in length. However, longer and shorter portions may be used. All or only a part of this narrow portion may be subcutaneously implanted into the patient. The lengths of other implantable sensors **42** will vary depending, at least in part, on the portion of the patient into which the sensor **42** is to be implanted or inserted.

Conductive Traces

At least one conductive trace **52** is formed on the substrate for use in constructing a working electrode **58**. In addition,

10

other conductive traces **52** may be formed on the substrate **50** for use as electrodes (e.g., additional working electrodes, as well as counter, counter/reference, and/or reference electrodes) and other components, such as a temperature probe. The conductive traces **52** may extend most of the distance along a length **57** of the sensor **50**, as illustrated in FIG. 2, although this is not necessary. The placement of the conductive traces **52** may depend on the particular configuration of the analyte monitoring system (e.g., the placement of control unit contacts and/or the sample chamber in relation to the sensor **42**). For implantable sensors, particularly subcutaneously implantable sensors, the conductive traces typically extend close to the tip of the sensor **42** to minimize the amount of the sensor that must be implanted.

The conductive traces **52** may be formed on the substrate **50** by a variety of techniques, including, for example, photolithography, screen printing, or other impact or non-impact printing techniques. The conductive traces **52** may also be formed by carbonizing conductive traces **52** in an organic (e.g., polymeric or plastic) substrate **50** using a laser. A description of some exemplary methods for forming the sensor **42** is provided in U.S. patent application Ser. No. 09/034,422, incorporated herein by reference.

Another method for disposing the conductive traces **52** on the substrate **50** includes the formation of recessed channels **54** in one or more surfaces of the substrate **50** and the subsequent filling of these recessed channels **54** with a conductive material **56**, as shown in FIG. 3A. The recessed channels **54** may be formed by indenting, embossing, or otherwise creating a depression in the surface of the substrate **50**. Exemplary methods for forming channels and electrodes in a surface of a substrate can be found in U.S. patent application Ser. No. 09/034,422. The depth of the channels is typically related to the thickness of the substrate **50**. In one embodiment, the channels have depths in the range of about 12.5 to 75 μm (0.5 to 3 mils), and preferably about 25 to 50 μm (1 to 2 mils).

The conductive traces are typically formed using a conductive material **56** such as carbon (e.g., graphite), a conductive polymer, a metal or alloy (e.g., gold or gold alloy), or a metallic compound (e.g., ruthenium dioxide or titanium dioxide). The formation of films of carbon, conductive polymer, metal, alloy, or metallic compound are well-known and include, for example, chemical vapor deposition (CVD), physical vapor deposition, sputtering, reactive sputtering, printing, coating, and painting. The conductive material **56** which fills the channels **54** is often formed using a precursor material, such as a conductive ink or paste. In these embodiments, the conductive material **56** is deposited on the substrate **50** using methods such as coating, painting, or applying the material using a spreading instrument, such as a coating blade. Excess conductive material between the channels **54** is then removed by, for example, running a blade along the substrate surface.

In one embodiment, the conductive material **56** is a part of a precursor material, such as a conductive ink, obtainable, for example, from Ercon, Inc. (Wareham, Mass.), Metech, Inc. (Elverson, Pa.), E.I. du Pont de Nemours and Co. (Wilmington, Del.), Emca-Remex Products (Montgomeryville, Pa.), or MCA Services (Melbourn, Great Britain). The conductive ink is typically applied as a semi-liquid or paste which contains particles of the carbon, metal, alloy, or metallic compound and a solvent or dispersant. After application of the conductive ink on the substrate **50** (e.g., in the channels **54**), the solvent or dispersant evaporates to leave behind a solid mass of conductive material **56**.

In addition to the particles of carbon, metal, alloy, or metallic compound, the conductive ink may also contain a

US 6,565,509 B1

11

binder. The binder may optionally be cured to further bind the conductive material **56** within the channel **54** and/or on the substrate **50**. Curing the binder increases the conductivity of the conductive material **56**. However, this is typically not necessary as the currents carried by the conductive material **56** within the conductive traces **52** are often relatively low (usually less than 1 μA and often less than 100 nA). Typical binders include, for example, polyurethane resins, cellulose derivatives, elastomers, and highly fluorinated polymers. Examples of elastomers include silicones, polymeric dienes, and acrylonitrile-butadiene-styrene (ABS) resins. One example of a fluorinated polymer binder is Teflon® (DuPont, Wilmington, Del.). These binders are cured using, for example, heat or light, including ultraviolet (UV) light. The appropriate curing method typically depends on the particular binder which is used.

Often, when a liquid or semiliquid precursor of the conductive material **56** (e.g., a conductive ink) is deposited in the channel **54**, the precursor fills the channel **54**. However, when the solvent or dispersant evaporates, the conductive material **56** which remains may lose volume such that the conductive material **56** may or may not continue to fill the channel **54**. Preferred conductive materials **56** do not pull away from the substrate **50** as they lose volume, but rather decrease in height within the channel **54**. These conductive materials **56** typically adhere well to the substrate **50** and therefore do not pull away from the substrate **50** during evaporation of the solvent or dispersant. Other suitable conductive materials **56** either adhere to at least a portion of the substrate **50** and/or contain another additive, such as a binder, which adheres the conductive material **56** to the substrate **50**. Preferably, the conductive material **56** in the channels **54** is non-leachable, and more preferably immobilized on the substrate **50**. In some embodiments, the conductive material **56** may be formed by multiple applications of a liquid or semiliquid precursor interspersed with removal of the solvent or dispersant.

In another embodiment, the channels **54** are formed using a laser. The laser carbonizes the polymer or plastic material. The carbon formed in this process is used as the conductive material **56**. Additional conductive material **56**, such as a conductive carbon ink, may be used to supplement the carbon formed by the laser.

In a further embodiment, the conductive traces **52** are formed by pad printing techniques. For example, a film of conductive material is formed either as a continuous film or as a coating layer deposited on a carrier film. This film of conductive material is brought between a print head and the substrate **50**. A pattern on the surface of the substrate **50** is made using the print head according to a desired pattern of conductive traces **52**. The conductive material is transferred by pressure and/or heat from the film of conductive material to the substrate **50**. This technique often produces channels (e.g., depressions caused by the print head) in the substrate **50**. Alternatively, the conductive material is deposited on the surface of the substrate **50** without forming substantial depressions.

In other embodiments, the conductive traces **52** are formed by non-impact printing techniques. Such techniques include electrophotography and magnetography. In these processes, an image of the conductive traces **52** is electrically or magnetically formed on a drum. A laser or LED may be used to electrically form an image. A magnetic recording head may be used to magnetically form an image. A toner material (e.g., a conductive material, such as a conductive ink) is then attracted to portions of the drum according to the image. The toner material is then applied to the substrate by

12

contact between the drum and the substrate. For example, the substrate may be rolled over the drum. The toner material may then be dried and/or a binder in the toner material may be cured to adhere the toner material to the substrate.

Another non-impact printing technique includes ejecting droplets of conductive material onto the substrate in a desired pattern. Examples of this technique include ink jet printing and piezo jet printing. An image is sent to the printer which then ejects the conductive material (e.g., a conductive ink) according to the pattern. The printer may provide a continuous stream of conductive material or the printer may eject the conductive material in discrete amounts at the desired points.

Yet another non-impact printing embodiment of forming the conductive traces includes an ionographic process. In this process, a curable, liquid precursor, such as a photopolymerizable acrylic resin (e.g., Solimer 7501 from Cubital, Bad Kreuznach, Germany) is deposited over a surface of a substrate **50**. A photomask having a positive or negative image of the conductive traces **52** is then used to cure the liquid precursor. Light (e.g., visible or ultraviolet light) is directed through the photomask to cure the liquid precursor and form a solid layer over the substrate according to the image on the photomask. Uncured liquid precursor is removed leaving behind channels **54** in the solid layer. These channels **54** can then be filled with conductive material **56** to form conductive traces **52**.

Conductive traces **52** (and channels **54**, if used) can be formed with relatively narrow widths, for example, in the range of 25 to 250 μm , and including widths of, for example, 250 μm , 150 μm , 100 μm , 75 μm , 50 μm , 25 μm or less by the methods described above. In embodiments with two or more conductive traces **52** on the same side of the substrate **50**, the conductive traces **52** are separated by distances sufficient to prevent conduction between the conductive traces **52**. The edge-to-edge distance between the conductive traces is preferably in the range of 25 to 250 μm and may be, for example, 150 μm , 100 μm , 75 μm , 50 μm , or less. The density of the conductive traces **52** on the substrate **50** is preferably in the range of about 150 to 700 $\mu\text{m}/\text{trace}$ and may be as small as 667 $\mu\text{m}/\text{trace}$ or less, 333 $\mu\text{m}/\text{trace}$ or less, or even 167 $\mu\text{m}/\text{trace}$ or less.

The working electrode **58** and the counter electrode **60** (if a separate reference electrode is used) are often made using a conductive material **56**, such as carbon. Suitable carbon conductive inks are available from Ercon, Inc. (Wareham, Mass.), Metech, Inc. (Elverson, Pa.), E.I. du Pont de Nemours and Co. (Wilmington, Del.), Emca-Remex Products (Montgomeryville, Pa.), or MCA Services (Melbourn, Great Britain). Typically, the working surface **51** of the working electrode **58** is at least a portion of the conductive trace **52** that is in contact with the analyte-containing fluid (e.g., implanted in the patient).

The reference electrode **62** and/or counter/reference electrode are typically formed using conductive material **56** that is a suitable reference material, for example silver/silver chloride or a non-leachable redox couple bound to a conductive material, for example, a carbon-bound redox couple. Suitable silver/silver chloride conductive inks are available from Ercon, Inc. (Wareham, Mass.), Metech, Inc. (Elverson, Pa.), E.I. du Pont de Nemours and Co. (Wilmington, Del.), Emca-Remex Products (Montgomeryville, Pa.), or MCA Services (Melbourn, Great Britain). Silver/silver chloride electrodes illustrate a type of reference electrode that involves the reaction of a metal electrode with a constituent of the sample or body fluid, in this case, Cl.

Suitable redox couples for binding to the conductive material of the reference electrode include, for example,

US 6,565,509 B1

13

redox polymers (e.g., polymers having multiple redox centers.) It is preferred that the reference electrode surface be non-corroding so that an erroneous potential is not measured. Preferred conductive materials include less corrosive metals, such as gold and palladium. Most preferred are non-corrosive materials including non-metallic conductors, such as carbon and conducting polymers. A redox polymer can be adsorbed on or covalently bound to the conductive material of the reference electrode, such as a carbon surface of a conductive trace **52**. Non-polymeric redox couples can be similarly bound to carbon or gold surfaces.

A variety of methods may be used to immobilize a redox polymer on an electrode surface. One method is adsorptive immobilization. This method is particularly useful for redox polymers with relatively high molecular weights. The molecular weight of a polymer may be increased, for example, by cross-linking.

Another method for immobilizing the redox polymer includes the functionalization of the electrode surface and then the chemical bonding, often covalently, of the redox polymer to the functional groups on the electrode surface. One example of this type of immobilization begins with a poly(4-vinylpyridine). The polymer's pyridine rings are, in part, complexed with a reducible/oxidizable species, such as $[\text{Os}(\text{bpy})_2\text{Cl}]^{+/2+}$ where bpy is 2,2'-bipyridine. Part of the pyridine rings are quaternized by reaction with 2-bromoethylamine. The polymer is then crosslinked, for example, using a diepoxide, such as polyethylene glycol diglycidyl ether.

Carbon surfaces can be modified for attachment of a redox species or polymer, for example, by electroreduction of a diazonium salt. As an illustration, reduction of a diazonium salt formed upon diazotization of p-aminobenzoic acid modifies a carbon surface with phenylcarboxylic acid functional groups. These functional groups can then be activated by a carbodiimide, such as 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride. The activated functional groups are then bound with an amine-functionalized redox couple, such as the quaternized osmium-containing redox polymer described above or 2-aminoethylferrocene, to form the redox couple.

Similarly, gold can be functionalized by an amine, such as cystamine. A redox couple such as $[\text{Os}(\text{bpy})_2(\text{pyridine-4-carboxylate})\text{Cl}]^{0/+}$ is activated by 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride to form a reactive O-acylisourea which reacts with the gold-bound amine to form an amide.

In one embodiment, in addition to using the conductive traces **52** as electrodes or probe leads, two or more of the conductive traces **52** on the substrate **50** are used to give the patient a mild electrical shock when, for example, the analyte level exceeds a threshold level. This shock may act as a warning or alarm to the patient to initiate some action to restore the appropriate level of the analyte.

The mild electrical shock is produced by applying a potential between any two conductive traces **52** that are not otherwise connected by a conductive path. For example, two of the electrodes **58**, **60**, **62** or one electrode **58**, **60**, **62** and the temperature probe **66** may be used to provide the mild shock. Preferably, the working electrode **58** and the reference electrode **62** are not used for this purpose as this may cause some damage to the chemical components on or proximate to the particular electrode (e.g., the sensing layer on the working electrode or the redox couple on the reference electrode).

The current used to produce the mild shock is typically 0.1 to 1 mA. Higher or lower currents may be used, although

14

care should be taken to avoid harm to the patient. The potential between the conductive traces is typically 1 to 10 volts. However, higher or lower voltages may be used depending, for example, on the resistance of the conductive traces **52**, the distance between the conductive traces **52** and the desired amount of current. When the mild shock is delivered, potentials at the working electrode **58** and across the temperature probe **66** may be removed to prevent harm to those components caused by unwanted conduction between the working electrode **58** (and/or temperature probe **66**, if used) and the conductive traces **52** which provide the mild shock.

Contact Pads

Typically, each of the conductive traces **52** includes a contact pad **49**. The contact pad **49** may simply be a portion of the conductive trace **52** that is indistinguishable from the rest of the trace **52** except that the contact pad **49** is brought into contact with the conductive contacts of a control unit (e.g., the sensor control unit **44** of FIG. 1). More commonly, however, the contact pad **49** is a region of the conductive trace **52** that has a larger width than other regions of the trace **52** to facilitate a connection with the contacts on the control unit. By making the contact pads **49** relatively large as compared with the width of the conductive traces **52**, the need for precise registration between the contact pads **49** and the contacts on the control unit is less critical than with small contact pads.

The contact pads **49** are typically made using the same material as the conductive material **56** of the conductive traces **52**. However, this is not necessary. Although metal, alloys, and metallic compounds may be used to form the contact pads **49**, in some embodiments, it is desirable to make the contact pads **49** from a carbon or other non-metallic material, such as a conducting polymer. In contrast to metal or alloy contact pads, carbon and other non-metallic contact pads are not easily corroded if the contact pads **49** are in a wet, moist, or humid environment. Metals and alloys may corrode under these conditions, particularly if the contact pads **49** and contacts of the control unit are made using different metals or alloys. However, carbon and non-metallic contact pads **49** do not significantly corrode, even if the contacts of the control device are metal or alloy.

One embodiment of the invention includes a sensor **42** having contact pads **49** and a control unit **44** having conductive contacts (not shown). During operation of the sensor **42**, the contact pads **49** and conductive contacts are in contact with each other. In this embodiment, either the contact pads **49** or the conductive contacts are made using a non-corroding, conductive material. Such materials include, for example, carbon and conducting polymers. Preferred non-corroding materials include graphite and vitreous carbon. The opposing contact pad or conductive contact is made using carbon, a conducting polymer, a metal, such as gold, palladium, or platinum group metal, or a metallic compound, such as ruthenium dioxide. This configuration of contact pads and conductive contacts typically reduces corrosion. Preferably, when the sensor is placed in a 3 mM, and more preferably, in a 100 mM, NaCl solution, the signal arising due to the corrosion of the contact pads and/or conductive contacts is less than 3% of the signal generated by the sensor when exposed to concentration of analyte in the normal physiological range. For at least some subcutaneous glucose sensors, the current generated by analyte in a normal physiological range ranges from 3 to 500 nA.

Each of the electrodes **58**, **60**, **62**, as well as the two probe leads **68**, **70** of the temperature probe **66** (described below), are connected to contact pads **49** as shown in FIGS. 10 and

US 6,565,509 B1

15

11. In one embodiment (not shown), the contact pads 49 are on the same side of the substrate 50 as the respective electrodes or temperature probe leads to which the contact pads 49 are attached.

In other embodiments, the conductive traces 52 on at least one side are connected through vias in the substrate to contact pads 49a on the opposite surface of the substrate 50, as shown in FIGS. 10 and 11. An advantage of this configuration is that contact between the contacts on the control unit and each of the electrodes 58, 60, 62 and the probe leads 68,70 of the temperature probe 66 can be made from a single side of the substrate 50.

In yet other embodiments (not shown), vias through the substrate are used to provide contact pads on both sides of the substrate 50 for each conductive trace 52. The vias connecting the conductive traces 52 with the contact pads 49a can be formed by making holes through the substrate 50 at the appropriate points and then filling the holes with conductive material 56.

Exemplary Electrode Configurations

A number of exemplary electrode configurations are described below, however, it will be understood that other configurations may also be used. In one embodiment, illustrated in FIG. 3A, the sensor 42 includes two working electrodes 58a, 58b and one counter electrode 60, which also functions as a reference electrode. In another embodiment, the sensor includes one working electrode 58a, one counter electrode 60, and one reference electrode 62, as shown in FIG. 3B. Each of these embodiments is illustrated with all of the electrodes formed on the same side of the substrate 50.

Alternatively, one or more of the electrodes may be formed on an opposing side of the substrate 50. This may be convenient if the electrodes are formed using two different types of conductive material 56 (e.g., carbon and silver/silver chloride). Then, at least in some embodiments, only one type of conductive material 56 needs to be applied to each side of the substrate 50, thereby reducing the number of steps in the manufacturing process and/or easing the registration constraints in the process. For example, if the working electrode 58 is formed using a carbon-based conductive material 56 and the reference or counter/reference electrode is formed using a silver/silver chloride conductive material 56, then the working electrode and reference or counter/reference electrode may be formed on opposing sides of the substrate 50 for ease of manufacture.

In another embodiment, two working electrodes 58 and one counter electrode 60 are formed on one side of the substrate 50 and one reference electrode 62 and a temperature probe 66 are formed on an opposing side of the substrate 50, as illustrated in FIG. 6. The opposing sides of the tip of this embodiment of the sensor 42 are illustrated in FIGS. 7 and 8.

Sensing Layer

Some analytes, such as oxygen, can be directly electrooxidized or electroreduced on the working electrode 58. Other analytes, such as glucose and lactate, require the presence of at least one electron transfer agent and/or at least one catalyst to facilitate the electrooxidation or electroreduction of the analyte. Catalysts may also be used for those analyte, such as oxygen, that can be directly electrooxidized or electroreduced on the working electrode 58. For these analytes, each working electrode 58 has a sensing layer 64 formed proximate to or on a working surface of the working electrode 58. Typically, the sensing layer 64 is formed near or on only a small portion of the working electrode 58, often near a tip of the sensor 42. This limits the amount of material needed to form the sensor 42 and places the sensing layer 64

16

in the best position for contact with the analyte-containing fluid (e.g., a body fluid, sample fluid, or carrier fluid).

The sensing layer 64 includes one or more components designed to facilitate the electrolysis of the analyte. The sensing layer 64 may include, for example, a catalyst to catalyze a reaction of the analyte and produce a response at the working electrode 58, an electron transfer agent to indirectly or directly transfer electrons between the analyte and the working electrode 58, or both.

The sensing layer 64 may be formed as a solid composition of the desired components (e.g., an electron transfer agent and/or a catalyst). These components are preferably non-leachable from the sensor 42 and more preferably are immobilized on the sensor 42. For example, the components may be immobilized on a working electrode 58. Alternatively, the components of the sensing layer 64 may be immobilized within or between one or more membranes or films disposed over the working electrode 58 or the components may be immobilized in a polymeric or sol-gel matrix. Examples of immobilized sensing layers are described in U.S. Pat. Nos. 5,262,035, 5,264,104, 5,264,105, 5,320,725, 5,593,852, and 5,665,222, U.S. patent application Ser. No. 08/540,789, and PCT Patent Application No. U.S. Ser. No. 98/02403 entitled "Soybean Peroxidase Electrochemical Sensor", filed on Feb. 11, 1998, incorporated herein by reference.

In some embodiments, one or more of the components of the sensing layer 64 may be solvated, dispersed, or suspended in a fluid within the sensing layer 64, instead of forming a solid composition. The fluid may be provided with the sensor 42 or may be absorbed by the sensor 42 from the analyte-containing fluid. Preferably, the components which are solvated, dispersed, or suspended in this type of sensing layer 64 are non-leachable from the sensing layer. Non-leachability may be accomplished, for example, by providing barriers (e.g., the electrode, substrate, membranes, and/or films) around the sensing layer which prevent the leaching of the components of the sensing layer 64. One example of such a barrier is a microporous membrane or film which allows diffusion of the analyte into the sensing layer 64 to make contact with the components of the sensing layer 64, but reduces or eliminates the diffusion of the sensing layer components (e.g., an electron transfer agent and/or a catalyst) out of the sensing layer 64.

A variety of different sensing layer configurations can be used. In one embodiment, the sensing layer 64 is deposited on the conductive material 56 of a working electrode 58a, as illustrated in FIGS. 3A and 3B. The sensing layer 64 may extend beyond the conductive material 56 of the working electrode 58a. In some cases, the sensing layer 64 may also extend over the counter electrode 60 or reference electrode 62 without degrading the performance of the glucose sensor. For those sensors 42 which utilize channels 54 within which the conductive material 56 is deposited, a portion of the sensing layer 64 may be formed within the channel 54 if the conductive material 56 does not fill the channel 54.

A sensing layer 64 in direct contact with the working electrode 58a may contain an electron transfer agent to transfer electrons directly or indirectly between the analyte and the working electrode, as well as a catalyst to facilitate a reaction of the analyte. For example, a glucose, lactate, or oxygen electrode may be formed having a sensing layer which contains a catalyst, such as glucose oxidase, lactate oxidase, or laccase, respectively, and an electron transfer agent that facilitates the electrooxidation of the glucose, lactate, or oxygen, respectively.

In another embodiment, the sensing layer 64 is not deposited directly on the working electrode 58a. Instead, the

US 6,565,509 B1

17

sensing layer **64** is spaced apart from the working electrode **58a**, as illustrated in FIG. **4A**, and separated from the working electrode **58a** by a separation layer **61**. The separation layer **61** typically includes one or more membranes or films. In addition to separating the working electrode **58a** from the sensing layer **64**, the separation layer **61** may also act as a mass transport limiting layer or an interferent eliminating layer, as described below.

Typically, a sensing layer **64**, which is not in direct contact with the working electrode **58a**, includes a catalyst that facilitates a reaction of the analyte. However, this sensing layer **64** typically does not include an electron transfer agent that transfers electrons directly from the working electrode **58a** to the analyte, as the sensing layer **64** is spaced apart from the working electrode **58a**. One example of this type of sensor is a glucose or lactate sensor which includes an enzyme (e.g., glucose oxidase or lactate oxidase, respectively) in the sensing layer **64**. The glucose or lactate reacts with a second compound (e.g., oxygen) in the presence of the enzyme. The second compound is then electrooxidized or electroreduced at the electrode. Changes in the signal at the electrode indicate changes in the level of the second compound in the fluid and are proportional to changes in glucose or lactate level and, thus, correlate to the analyte level.

In another embodiment, two sensing layers **63**, **64** are used, as shown in FIG. **4B**. Each of the two sensing layers **63**, **64** may be independently formed on the working electrode **58a** or in proximity to the working electrode **58a**. One sensing layer **64** is typically, although not necessarily, spaced apart from the working electrode **58a**. For example, this sensing layer **64** may include a catalyst which catalyzes a reaction of the analyte to form a product compound. The product compound is then electrolyzed in the second sensing layer **63** which may include an electron transfer agent to transfer electrons between the working electrode **58a** and the product compound and/or a second catalyst to catalyze a reaction of the product compound to generate a signal at the working electrode **58a**.

For example, a glucose or lactate sensor may include a first sensing layer **64** which is spaced apart from the working electrode and contains an enzyme, for example, glucose oxidase or lactate oxidase. The reaction of glucose or lactate in the presence of the appropriate enzyme forms hydrogen peroxide. A second sensing layer **63** is provided directly on the working electrode **58a** and contains a peroxidase enzyme and an electron transfer agent to generate a signal at the electrode in response to the hydrogen peroxide. The level of hydrogen peroxide indicated by the sensor then correlates to the level of glucose or lactate. Another sensor which operates similarly can be made using a single sensing layer with both the glucose or lactate oxidase and the peroxidase being deposited in the single sensing layer. Examples of such sensors are described in U.S. Pat. No. 5,593,852, U.S. patent application Ser. No. 08/540,789, and PCT Patent Application No. U.S. Ser. No. 98/02403 entitled "Soybean Peroxidase Electrochemical Sensor", filed on Feb. 11, 1998, incorporated herein by reference.

In some embodiments, one or more of the working electrodes **58b** do not have a corresponding sensing layer **64**, as shown in FIGS. **3A** and **4A**, or have a sensing layer (not shown) which does not contain one or more components (e.g., an electron transfer agent or catalyst) needed to electrolyze the analyte. The signal generated at this working electrode **58b** typically arises from interferents and other sources, such as ions, in the fluid, and not in response to the analyte (because the analyte is not electrooxidized or

18

electroreduced). Thus, the signal at this working electrode **58b** corresponds to a background signal. The background signal can be removed from the analyte signal obtained from other working electrodes **58a** that are associated with fully-functional sensing layers **64** by, for example, subtracting the signal at working electrode **58b** from the signal at working electrode **58a**.

Sensors having multiple working electrodes **58a** may also be used to obtain more precise results by averaging the signals or measurements generated at these working electrodes **58a**. In addition, multiple readings at a single working electrode **58a** or at multiple working electrodes may be averaged to obtain more precise data.

Electron Transfer Agent

In many embodiments, the sensing layer **64** contains one or more electron transfer agents in contact with the conductive material **56** of the working electrode **58**, as shown in FIGS. **3A** and **3B**. In some embodiments of the invention, there is little or no leaching of the electron transfer agent away from the working electrode **58** during the period in which the sensor **42** is implanted in the patient. A diffusing or leachable (i.e., releasable) electron transfer agent often diffuses into the analyte-containing fluid, thereby reducing the effectiveness of the electrode by reducing the sensitivity of the sensor over time. In addition, a diffusing or leaching electron transfer agent in an implantable sensor **42** may also cause damage to the patient. In these embodiments, preferably, at least 90%, more preferably, at least 95%, and, most preferably, at least 99%, of the electron transfer agent remains disposed on the sensor after immersion in the analyte-containing fluid for 24 hours, and, more preferably, for 72 hours. In particular, for an implantable sensor, preferably, at least 90%, more preferably, at least 95%, and most preferably, at least 99%, of the electron transfer agent remains disposed on the sensor after immersion in the body fluid at 37° C. for 24 hours, and, more preferably, for 72 hours.

In some embodiments of the invention, to prevent leaching, the electron transfer agents are bound or otherwise immobilized on the working electrode **58** or between or within one or more membranes or films disposed over the working electrode **58**. The electron transfer agent may be immobilized on the working electrode **58** using, for example, a polymeric or sol-gel immobilization technique. Alternatively, the electron transfer agent may be chemically (e.g., ionically, covalently, or coordinatively) bound to the working electrode **58**, either directly or indirectly through another molecule, such as a polymer, that is in turn bound to the working electrode **58**.

Application of the sensing layer **64** on a working electrode **58a** is one method for creating a working surface for the working electrode **58a**, as shown in FIGS. **3A** and **3B**. The electron transfer agent mediates the transfer of electrons to electrooxidize or electroreduce an analyte and thereby permits a current flow between the working electrode **58** and the counter electrode **60** via the analyte. The mediation of the electron transfer agent facilitates the electrochemical analysis of analytes which are not suited for direct electrochemical reaction on an electrode.

In general, the preferred electron transfer agents are electroreducible and electrooxidizable ions or molecules having redox potentials that are a few hundred millivolts above or below the redox potential of the standard calomel electrode (SCE). Preferably, the electron transfer agents are not more reducing than about -150 mV and not more oxidizing than about +400 mV versus SCE.

The electron transfer agent may be organic, organometallic, or inorganic. Examples of organic redox

US 6,565,509 B1

19

species are quinones and species that in their oxidized state have quinoid structures, such as Nile blue and indophenol. Some quinones and partially oxidized quinhydrones react with functional groups of proteins such as the thiol groups of cysteine, the amine groups of lysine and arginine, and the phenolic groups of tyrosine which may render those redox species unsuitable for some of the sensors of the present invention because of the presence of the interfering proteins in an analyte-containing fluid. Usually substituted quinones and molecules with quinoid structure are less reactive with proteins and are preferred. A preferred tetrasubstituted quinone usually has carbon atoms in positions 1, 2, 3, and 4.

In general, electron transfer agents suitable for use in the invention have structures or charges which prevent or substantially reduce the diffusional loss of the electron transfer agent during the period of time that the sample is being analyzed. The preferred electron transfer agents include a redox species bound to a polymer which can in turn be immobilized on the working electrode. The bond between the redox species and the polymer may be covalent, coordinative, or ionic. Useful electron transfer agents and methods for producing them are described in U.S. Pat. Nos. 5,264,104; 5,356,786; 5,262,035; and 5,320,725, incorporated herein by reference. Although any organic or organo-metallic redox species can be bound to a polymer and used as an electron transfer agent, the preferred redox species is a transition metal compound or complex. The preferred transition metal compounds or complexes include osmium, ruthenium, iron, and cobalt compounds or complexes. The most preferred are osmium compounds and complexes. It will be recognized that many of the redox species described below may also be used, typically without a polymeric component, as electron transfer agents in a carrier fluid or in a sensing layer of a sensor where leaching of the electron transfer agent is acceptable.

One type of non-releasable polymeric electron transfer agent contains a redox species covalently bound in a polymeric composition. An example of this type of mediator is poly(vinylferrocene).

Another type of non-releasable electron transfer agent contains an ionically-bound redox species. Typically, this type of mediator includes a charged polymer coupled to an oppositely charged redox species. Examples of this type of mediator include a negatively charged polymer such as Nafion® (DuPont) coupled to a positively charged redox species such as an osmium or ruthenium polypyridyl cation. Another example of an ionically-bound mediator is a positively charged polymer such as quaternized poly(4-vinyl pyridine) or poly(1-vinyl imidazole) coupled to a negatively charged redox species such as ferricyanide or ferrocyanide. The preferred ionically-bound redox species is a highly charged redox species bound within an oppositely charged redox polymer.

In another embodiment of the invention, suitable non-releasable electron transfer agents include a redox species coordinatively bound to a polymer. For example, the mediator may be formed by coordination of an osmium or cobalt 2,2'-bipyridyl complex to poly(1-vinyl imidazole) or poly(4-vinyl pyridine).

The preferred electron transfer agents are osmium transition metal complexes with one or more ligands, each ligand having a nitrogen-containing heterocycle such as 2,2'-bipyridine, 1,10-phenanthroline, or derivatives thereof. Furthermore, the preferred electron transfer agents also have one or more ligands covalently bound in a polymer, each ligand having at least one nitrogen-containing heterocycle, such as pyridine, imidazole, or derivatives thereof. These

20

preferred electron transfer agents exchange electrons rapidly between each other and the working electrodes 58 so that the complex can be rapidly oxidized and reduced.

One example of a particularly useful electron transfer agent includes (a) a polymer or copolymer having pyridine or imidazole functional groups and (b) osmium cations complexed with two ligands, each ligand containing 2,2'-bipyridine, 1,10-phenanthroline, or derivatives thereof, the two ligands not necessarily being the same. Preferred derivatives of 2,2'-bipyridine for complexation with the osmium cation are 4,4'-dimethyl-2,2'-bipyridine and mono-, di-, and polyalkoxy-2,2'-bipyridines, such as 4,4'-dimethoxy-2,2'-bipyridine. Preferred derivatives of 1,10-phenanthroline for complexation with the osmium cation are 4,7-dimethyl-1,10-phenanthroline and mono-, di-, and polyalkoxy-1,10-phenanthrolines, such as 4,7-dimethoxy-1,10-phenanthroline. Preferred polymers for complexation with the osmium cation include polymers and copolymers of poly(1-vinyl imidazole) (referred to as "PVI") and poly(4-vinyl pyridine) (referred to as "PVP"). Suitable copolymer substituents of poly(1-vinyl imidazole) include acrylonitrile, acrylamide, and substituted or quaternized N-vinyl imidazole. Most preferred are electron transfer agents with osmium complexed to a polymer or copolymer of poly(1-vinyl imidazole).

The preferred electron transfer agents have a redox potential ranging from -100 mV to about +150 mV versus the standard calomel electrode (SCE). Preferably, the potential of the electron transfer agent ranges from -100 mV to +150 mV and more preferably, the potential ranges from -50 mV to +50 V. The most preferred electron transfer agents have osmium redox centers and a redox potential ranging from +50 V to -150 mV versus SCE.

Catalyst

The sensing layer 64 may also include a catalyst which is capable of catalyzing a reaction of the analyte. The catalyst may also, in some embodiments, act as an electron transfer agent. One example of a suitable catalyst is an enzyme which catalyzes a reaction of the analyte. For example, a catalyst, such as a glucose oxidase, glucose dehydrogenase (e.g., pyrroloquinoline quinone glucose dehydrogenase (PQQ)), or oligosaccharide dehydrogenase, may be used when the analyte is glucose. A lactate oxidase or lactate dehydrogenase may be used when the analyte is lactate. Laccase may be used when the analyte is oxygen or when oxygen is generated or consumed in response to a reaction of the analyte.

Preferably, the catalyst is non-leachably disposed on the sensor, whether the catalyst is part of a solid sensing layer in the sensor or solvated in a fluid within the sensing layer. More preferably, the catalyst is immobilized within the sensor (e.g., on the electrode and/or within or between a membrane or film) to prevent unwanted leaching of the catalyst away from the working electrode 58 and into the patient. This may be accomplished, for example, by attaching the catalyst to a polymer, cross linking the catalyst with another electron transfer agent (which, as described above, can be polymeric), and/or providing one or more barrier membranes or films with pore sizes smaller than the catalyst.

As described above, a second catalyst may also be used. This second catalyst is often used to catalyze a reaction of a product compound resulting from the catalyzed reaction of the analyte. The second catalyst typically operates with an electron transfer agent to electrolyze the product compound to generate a signal at the working electrode. Alternatively, the second catalyst may be provided in an interferent-eliminating layer to catalyze reactions that remove interferents, as described below.

US 6,565,509 B1

21

One embodiment of the invention is an electrochemical sensor in which the catalyst is mixed or dispersed in the conductive material **56** which forms the conductive trace **52** of a working electrode **58**. This may be accomplished, for example, by mixing a catalyst, such as an enzyme, in a carbon ink and applying the mixture into a channel **54** on the surface of the substrate **50**. Preferably, the catalyst is immobilized in the channel **53** so that it can not leach away from the working electrode **58**. This may be accomplished, for example, by curing a binder in the carbon ink using a curing technique appropriate to the binder. Curing techniques include, for example, evaporation of a solvent or dispersant, exposure to ultraviolet light, or exposure to heat. Typically, the mixture is applied under conditions that do not substantially degrade the catalyst. For example, the catalyst may be an enzyme that is heat-sensitive. The enzyme and conductive material mixture should be applied and cured, preferably, without sustained periods of heating. The mixture may be cured using evaporation or UV curing techniques or by the exposure to heat that is sufficiently short that the catalyst is not substantially degraded.

Another consideration for in vivo analyte sensors is the thermostability of the catalyst. Many enzymes have only limited stability at biological temperatures. Thus, it may be necessary to use large amounts of the catalyst and/or use a catalyst that is thermostable at the necessary temperature (e.g., 37° C. or higher for normal body temperature). A thermostable catalyst may be defined as a catalyst which loses less than 5% of its activity when held at 37° C. for at least one hour, preferably, at least one day, and more preferably at least three days. One example of a thermostable catalyst is soybean peroxidase. This particular thermostable catalyst may be used in a glucose or lactate sensor when combined either in the same or separate sensing layers with glucose or lactate oxidase or dehydrogenase. A further description of thermostable catalysts and their use in electrochemical inventions is found in U.S. Pat. No. 5,665,222 U.S. patent application Ser. No. 08/540,789, and PCT Application No. U.S. Ser. No. 98/02403 entitled "Soybean Peroxidase Electrochemical Sensor", filed on Feb. 11, 1998, Electrolysis of the Analyte

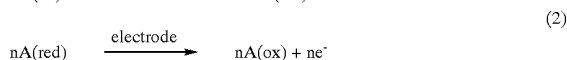
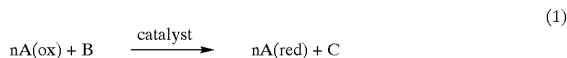
To electrolyze the analyte, a potential (versus a reference potential) is applied across the working and counter electrodes **58**, **60**. The minimum magnitude of the applied potential is often dependent on the particular electron transfer agent, analyte (if the analyte is directly electrolyzed at the electrode), or second compound (if a second compound, such as oxygen or hydrogen peroxide, whose level is dependent on the analyte level, is directly electrolyzed at the electrode). The applied potential usually equals or is more oxidizing or reducing, depending on the desired electrochemical reaction, than the redox potential of the electron transfer agent, analyte, or second compound, whichever is directly electrolyzed at the electrode. The potential at the working electrode is typically large enough to drive the electrochemical reaction to or near completion.

The magnitude of the potential may optionally be limited to prevent significant (as determined by the current generated in response to the analyte) electrochemical reaction of interferents, such as urate, ascorbate, and acetaminophen. The limitation of the potential may be obviated if these interferents have been removed in another way, such as by providing an interferent-limiting barrier, as described below, or by including a working electrode **58b** (see FIG. 3A) from which a background signal may be obtained.

When a potential is applied between the working electrode **58** and the counter electrode **60**, an electrical current

22

will flow. The current is a result of the electrolysis of the analyte or a second compound whose level is affected by the analyte. In one embodiment, the electrochemical reaction occurs via an electron transfer agent and the optional catalyst. Many analytes B are oxidized (or reduced) to products C by an electron transfer agent species A in the presence of an appropriate catalyst (e.g., an enzyme). The electron transfer agent A is then oxidized (or reduced) at the electrode. Electrons are collected by (or removed from) the electrode and the resulting current is measured. This process is illustrated by reaction equations (1) and (2) (similar equations may be written for the reduction of the analyte B by a redox mediator A in the presence of a catalyst):

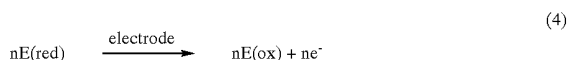


As an example, an electrochemical sensor may be based on the reaction of a glucose molecule with two non-leachable ferriocyanide anions in the presence of glucose oxidase to produce two non-leachable ferrocyanide anions, two hydrogen ions, and gluconolactone. The amount of glucose present is assayed by electrooxidizing the non-leachable ferrocyanide anions to non-leachable ferriocyanide anions and measuring the current.

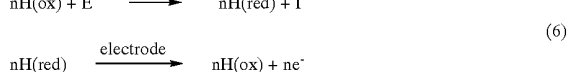
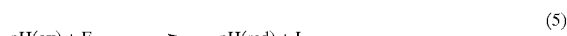
In another embodiment, a second compound whose level is affected by the analyte is electrolyzed at the working electrode. In some cases, the analyte D and the second compound, in this case, a reactant compound E, such as oxygen, react in the presence of the catalyst, as shown in reaction equation (3).



The reactant compound E is then directly oxidized (or reduced) at the working electrode, as shown in reaction equation (4)



Alternatively, the reactant compound E is indirectly oxidized (or reduced) using an electron transfer agent H (optionally in the presence of a catalyst), that is subsequently reduced or oxidized at the electrode, as shown in reaction equations (5) and (6).



In either case, changes in the concentration of the reactant compound, as indicated by the signal at the working electrode, correspond inversely to changes in the analyte (i.e., as the level of analyte increase then the level of reactant compound and the signal at the electrode decreases.)

In other embodiments, the relevant second compound is a product compound F, as shown in reaction equation (3). The product compound F is formed by the catalyzed reaction of analyte D and then be directly electrolyzed at the electrode

US 6,565,509 B1

23

or indirectly electrolyzed using an electron transfer agent and, optionally, a catalyst. In these embodiments, the signal arising from the direct or indirect electrolysis of the product compound F at the working electrode corresponds directly to the level of the analyte (unless there are other sources of the product compound). As the level of analyte increases, the level of the product compound and signal at the working electrode increases.

Those skilled in the art will recognize that there are many different reactions that will achieve the same result; namely the electrolysis of an analyte or a compound whose level depends on the level of the analyte. Reaction equations (1) through (6) illustrate non-limiting examples of such reactions.

Temperature Probe

A variety of optional items may be included in the sensor. One optional item is a temperature probe 66 (FIGS. 8 and 11). The temperature probe 66 may be made using a variety of known designs and materials. One exemplary temperature probe 66 is formed using two probe leads 68, 70 connected to each other through a temperature-dependent element 72 that is formed using a material with a temperature-dependent characteristic. An example of a suitable temperature-dependent characteristic is the resistance of the temperature-dependent element 72.

The two probe leads 68, 70 are typically formed using a metal, an alloy, a semimetal, such as graphite, a degenerate or highly doped semiconductor, or a small-band gap semiconductor. Examples of suitable materials include gold, silver, ruthenium oxide, titanium nitride, titanium dioxide, indium doped tin oxide, tin doped indium oxide, or graphite. The temperature-dependent element 72 is typically made using a fine trace (e.g., a conductive trace that has a smaller cross-section than that of the probe leads 68, 70) of the same conductive material as the probe leads, or another material such as a carbon ink, a carbon fiber, or platinum, which has a temperature-dependent characteristic, such as resistance, that provides a temperature-dependent signal when a voltage source is attached to the two probe leads 68, 70 of the temperature probe 66. The temperature-dependent characteristic of the temperature-dependent element 72 may either increase or decrease with temperature. Preferably, the temperature dependence of the characteristic of the temperature-dependent element 72 is approximately linear with temperature over the expected range of biological temperatures (about 25 to 45° C.), although this is not required.

Typically, a signal (e.g., a current) having an amplitude or other property that is a function of the temperature can be obtained by providing a potential across the two probe leads 68, 70 of the temperature probe 66. As the temperature changes, the temperature-dependent characteristic of the temperature-dependent element 72 increases or decreases with a corresponding change in the signal amplitude. The signal from the temperature probe 66 (e.g., the amount of current flowing through the probe) may be combined with the signal obtained from the working electrode 58 by, for example, scaling the temperature probe signal and then adding or subtracting the scaled temperature probe signal from the signal at the working electrode 58. In this manner, the temperature probe 66 can provide a temperature adjustment for the output from the working electrode 58 to offset the temperature dependence of the working electrode 58.

One embodiment of the temperature probe includes probe leads 68, 70 formed as two spaced-apart channels with a temperature-dependent element 72 formed as a cross-channel connecting the two spaced-apart channels, as illustrated in FIG. 8. The two spaced-apart channels contain a

24

conductive material, such as a metal, alloy, semimetal, degenerate semiconductor, or metallic compound. The cross-channel may contain the same material (provided the cross-channel has a smaller cross-section than the two spaced-apart channels) as the probe leads 68, 70. In other embodiments, the material in the cross-channel is different than the material of the probe leads 68, 70.

One exemplary method for forming this particular temperature probe includes forming the two spaced-apart channels and then filling them with the metallic or alloyed conductive material. Next, the cross-channel is formed and then filled with the desired material. The material in the cross-channel overlaps with the conductive material in each of the two spaced-apart channels to form an electrical connection.

For proper operation of the temperature probe 66, the temperature-dependent element 72 of the temperature probe 66 can not be shorted by conductive material formed between the two probe leads 68, 70. In addition, to prevent conduction between the two probe leads 68, 70 by ionic species within the body or sample fluid, a covering may be provided over the temperature-dependent element 72, and preferably over the portion of the probe leads 68, 70 that is implanted in the patient. The covering may be, for example, a non-conducting film disposed over the temperature-dependent element 72 and probe leads 68, 70 to prevent the ionic conduction. Suitable non-conducting films include, for example, Kapton™ polyimide films (DuPont, Wilmington, Del.).

Another method for eliminating or reducing conduction by ionic species in the body or sample fluid is to use an ac voltage source connected to the probe leads 68, 70. In this way, the positive and negative ionic species are alternately attracted and repelled during each half cycle of the ac voltage. This results in no net attraction of the ions in the body or sample fluid to the temperature probe 66. The maximum amplitude of the ac current through the temperature-dependent element 72 may then be used to correct the measurements from the working electrodes 58.

The temperature probe can be placed on the same substrate as the electrodes. Alternatively, a temperature probe may be placed on a separate substrate. In addition, the temperature probe may be used by itself or in conjunction with other devices.

Another embodiment of a temperature probe utilizes the temperature dependence of the conductivity of a solution (e.g., blood or interstitial fluid). Typically, the conductivity of an electrolyte-containing solution is dependent on the temperature of the solution, assuming that the concentration of electrolytes is relatively constant. Blood, interstitial fluid, and other bodily fluids are solutions with relatively constant levels of electrolytes. Thus, a sensor 42 can include two or more conductive traces (not shown) which are spaced apart by a known distance. A portion of these conductive traces is exposed to the solution and the conductivity between the exposed portions of the conductive traces is measured using known techniques (e.g., application of a constant or known current or potential and measurement of the resulting potential or current, respectively, to determine the conductivity).

A change in conductivity is related to a change in temperature. This relation can be modeled using linear, quadratic, exponential, or other relations. The parameters for this relationship typically do not vary significantly between most people. The calibration for the temperature probe can be determined by a variety of methods, including, for example, calibration of each sensor 42 using an independent method of determining temperature (e.g., a thermometer, an

US 6,565,509 B1

25

optical or electrical temperature detector, or the temperature probe 66, described above) or calibrating one sensor 42 and using that calibration for all other sensors in a batch based on uniformity in geometry.

Biocompatible Layer

An optional film layer 75 is formed over at least that portion of the sensor 42 which is subcutaneously inserted into the patient, as shown in FIG. 9. This optional film layer 74 may serve one or more functions. The film layer 74 prevents the penetration of large biomolecules into the electrodes. This is accomplished by using a film layer 74 having a pore size that is smaller than the biomolecules that are to be excluded. Such biomolecules may foul the electrodes and/or the sensing layer 64 thereby reducing the effectiveness of the sensor 42 and altering the expected signal amplitude for a given analyte concentration. The fouling of the working electrodes 58 may also decrease the effective life of the sensor 42. The biocompatible layer 74 may also prevent protein adhesion to the sensor 42, formation of blood clots, and other undesirable interactions between the sensor 42 and body.

For example, the sensor may be completely or partially coated on its exterior with a biocompatible coating. A preferred biocompatible coating is a hydrogel which contains at least 20 wt. % fluid when in equilibrium with the analyte-containing fluid. Examples of suitable hydrogels are described in U.S. Pat. No. 5,593,852, incorporated herein by reference, and include crosslinked polyethylene oxides, such as polyethylene oxide tetraacrylate.

Interferent-Eliminating Layer

An interferent-eliminating layer (not shown) may be included in the sensor 42. The interferent-eliminating layer may be incorporated in the biocompatible layer 75 or in the mass transport limiting layer 74 (described below) or may be a separate layer. Interferents are molecules or other species that are electroreduced or electrooxidized at the electrode, either directly or via an electron transfer agent, to produce a false signal. In one embodiment, a film or membrane prevents the penetration of one or more interferents into the region around the working electrodes 58. Preferably, this type of interferent-eliminating layer is much less permeable to one or more of the interferents than to the analyte.

The interferent-eliminating layer may include ionic components, such as Nafion®, incorporated into a polymeric matrix to reduce the permeability of the interferent-eliminating layer to ionic interferents having the same charge as the ionic components. For example, negatively charged compounds or compounds that form negative ions may be incorporated in the interferent-eliminating layer to reduce the permeation of negative species in the body or sample fluid.

Another example of an interferent-eliminating layer includes a catalyst for catalyzing a reaction which removes interferents. One example of such a catalyst is a peroxidase. Hydrogen peroxide reacts with interferents, such as acetaminophen, urate, and ascorbate. The hydrogen peroxide may be added to the analyte-containing fluid or may be generated in situ, by, for example, the reaction of glucose or lactate in the presence of glucose oxidase or lactate oxidase, respectively. Examples of interferent eliminating layers include a peroxidase enzyme crosslinked (a) using gluteraldehyde as a crosslinking agent or (b) oxidation of oligosaccharide groups in the peroxidase glycoenzyme with NaIO₄, followed by coupling of the aldehydes formed to hydrazide groups in a polyacrylamide matrix to form hydrazones are describe in U.S. Pat. Nos. 5,262,305 and 5,356,786, incorporated herein by reference.

26

Mass Transport Limiting Layer

A mass transport limiting layer 74 may be included with the sensor to act as a diffusion-limiting barrier to reduce the rate of mass transport of the analyte, for example, glucose or lactate, into the region around the working electrodes 58. By limiting the diffusion of the analyte, the steady state concentration of the analyte in the proximity of the working electrode 58 (which is proportional to the concentration of the analyte in the body or sample fluid) can be reduced. This extends the upper range of analyte concentrations that can still be accurately measured and may also expand the range in which the current increases approximately linearly with the level of the analyte.

It is preferred that the permeability of the analyte through the film layer 74 vary little or not at all with temperature, so as to reduce or eliminate the variation of current with temperature. For this reason, it is preferred that in the biologically relevant temperature range from about 25° C. to about 45° C., and most importantly from 30° C. to 40° C., neither the size of the pores in the film nor its hydration or swelling change excessively. Preferably, the mass transport limiting layer is made using a film that absorbs less than 5 wt. % of fluid over 24 hours. This may reduce or obviate any need for a temperature probe. For implantable sensors, it is preferable that the mass transport limiting layer is made using a film that absorbs less than 5 wt. % of fluid over 24 hours at 37° C.

Particularly useful materials for the film layer 74 are membranes that do not swell in the analyte-containing fluid that the sensor tests. Suitable membranes include 3 to 20,000 nm diameter pores. Membranes having 5 to 500 nm diameter pores with well-defined, uniform pore sizes and high aspect ratios are preferred. In one embodiment, the aspect ratio of the pores is preferably two or greater and more preferably five or greater.

Well-defined and uniform pores can be made by track etching a polymeric membrane using accelerated electrons, ions, or particles emitted by radioactive nuclei. Most preferred are anisotropic, polymeric, track etched membranes that expand less in the direction perpendicular to the pores than in the direction of the pores when heated. Suitable polymeric membranes included polycarbonate membranes from Poretics (Livermore, Calif., catalog number 19401, 0.01 μm pore size polycarbonate membrane) and Corning Costar Corp. (Cambridge, Mass., Nucleopore™ brand membranes with 0.015 μm pore size). Other polyolefin and polyester films may be used. It is preferred that the permeability of the mass transport limiting membrane changes no more than 4%, preferably, no more than 3%, and, more preferably, no more than 2%, per ° C. in the range from 30° C. to 40° C. when the membranes resides in the subcutaneous interstitial fluid.

In some embodiments of the invention, the mass transport limiting layer 74 may also limit the flow of oxygen into the sensor 42. This can improve the stability of sensors 42 that are used in situations where variation in the partial pressure of oxygen causes non-linearity in sensor response. In these embodiments, the mass transport limiting layer 74 restricts oxygen transport by at least 40%, preferably at least 60%, and more preferably at least 80%, than the membrane restricts transport of the analyte. For a given type of polymer, films having a greater density (e.g., a density closer to that of the crystalline polymer) are preferred. Polyesters, such as polyethylene terephthalate, are typically less permeable to oxygen and are, therefore, preferred over polycarbonate membranes.

US 6,565,509 B1

27

Anticlotting Agent

An implantable sensor may also, optionally, have an anticlotting agent disposed on a portion the substrate which is implanted into a patient. This anticlotting agent may reduce or eliminate the clotting of blood or other body fluid around the sensor, particularly after insertion of the sensor. Blood clots may foul the sensor or irreproducibly reduce the amount of analyte which diffuses into the sensor. Examples of useful anticlotting agents include heparin and tissue plasminogen activator (TPA), as well as other known anti-

clotting agents. The anticlotting agent may be applied to at least a portion of that part of the sensor 42 that is to be implanted. The anticlotting agent may be applied, for example, by bath, spraying, brushing, or dipping. The anticlotting agent is allowed to dry on the sensor 42. The anticlotting agent may be immobilized on the surface of the sensor or it may be allowed to diffuse away from the sensor surface. Typically, the quantities of anticlotting agent disposed on the sensor are far below the amounts typically used for treatment of medical conditions involving blood clots and, therefore, have only a limited, localized effect.

Sensor Lifetime

The sensor 42 may be designed to be a replaceable component in an in vivo analyte monitor, and particularly in an implantable analyte monitor. Typically, the sensor 42 is capable of operation over a period of days. Preferably, the period of operation is at least one day, more preferably at least three days, and most preferably at least one week. The sensor 42 can then be removed and replaced with a new sensor. The lifetime of the sensor 42 may be reduced by the fouling of the electrodes or by the leaching of the electron transfer agent or catalyst. These limitations on the longevity of the sensor 42 can be overcome by the use of a biocompatible layer 75 or non-leachable electron transfer agent and catalyst, respectively, as described above.

Another primary limitation on the lifetime of the sensor 42 is the temperature stability of the catalyst. Many catalysts are enzymes, which are very sensitive to the ambient temperature and may degrade at temperatures of the patient's body (e.g., approximately 37° C. for the human body). Thus, robust enzymes should be used where available. The sensor 42 should be replaced when a sufficient amount of the enzyme has been deactivated to introduce an unacceptable amount of error in the measurements.

Insertion Device

An insertion device 120 can be used to subcutaneously insert the sensor 42 into the patient, as illustrated in FIG. 12. The insertion device 120 is typically formed using structurally rigid materials, such as metal or rigid plastic. Preferred materials include stainless steel and ABS (acrylonitrile-butadiene-styrene) plastic. In some embodiments, the insertion device 120 is pointed and/or sharp at the tip 121 to facilitate penetration of the skin of the patient. A sharp, thin insertion device may reduce pain felt by the patient upon insertion of the sensor 42. In other embodiments, the tip 121 of the insertion device 120 has other shapes, including a blunt or flat shape. These embodiments may be particularly useful when the insertion device 120 does not penetrate the skin but rather serves as a structural support for the sensor 42 as the sensor 42 is pushed into the skin.

The insertion device 120 may have a variety of cross-sectional shapes, as shown in FIGS. 13A, 13B, and 13C. The insertion device 120 illustrated in FIG. 13A is a flat, planar, pointed strip of rigid material which may be attached or otherwise coupled to the sensor 42 to ease insertion of the sensor 42 into the skin of the patient, as well as to provide

28

structural support to the sensor 42 during insertion. The insertion devices 120 of FIGS. 13B and 13C are U- or V-shaped implements that support the sensor 42 to limit the amount that the sensor 42 may bend or bow during insertion. The cross-sectional width 124 of the insertion devices 120 illustrated in FIGS. 13B and 13C is typically 1 mm or less, preferably 700 μ m or less, more preferably 500 μ m or less, and most preferably 300 μ m or less. The cross-sectional height 126 of the insertion device 120 illustrated in FIGS. 13B and 13C is typically about 1 mm or less, preferably about 700 μ m or less, and more preferably about 500 μ m or less.

The sensor 42 itself may include optional features to facilitate insertion. For example, the sensor 42 may be pointed at the tip 123 to ease insertion, as illustrated in FIG. 12. In addition, the sensor 42 may include a barb 125 which helps retain the sensor 42 in the subcutaneous tissue of the patient. The barb 125 may also assist in anchoring the sensor 42 within the subcutaneous tissue of the patient during operation of the sensor 42. However, the barb 125 is typically small enough that little damage is caused to the subcutaneous tissue when the sensor 42 is removed for replacement. The sensor 42 may also include a notch 127 that can be used in cooperation with a corresponding structure (not shown) in the insertion device to apply pressure against the sensor 42 during insertion, but disengage as the insertion device 120 is removed. One example of such a structure in the insertion device is a rod (not shown) between two opposing sides of an insertion device 120 and at an appropriate height of the insertion device 120.

In operation, the sensor 42 is placed within or next to the insertion device 120 and then a force is provided against the insertion device 120 and/or sensor 42 to carry the sensor 42 into the skin of the patient. In one embodiment, the force is applied to the sensor 42 to push the sensor into the skin, while the insertion device 120 remains stationary and provides structural support to the sensor 42. Alternatively, the force is applied to the insertion device 120 and optionally to the sensor 42 to push a portion of both the sensor 42 and the insertion device 120 through the skin of the patient and into the subcutaneous tissue. The insertion device 120 is optionally pulled out of the skin and subcutaneous tissue with the sensor 42 remaining in the subcutaneous tissue due to frictional forces between the sensor 42 and the patient's tissue. If the sensor 42 includes the optional barb 125, then this structure may also facilitate the retention of the sensor 42 within the interstitial tissue as the barb catches in the tissue.

The force applied to the insertion device 120 and/or the sensor 42 may be applied manually or mechanically. Preferably, the sensor 42 is reproducibly inserted through the skin of the patient. In one embodiment, an insertion gun is used to insert the sensor. One example of an insertion gun 200 for inserting a sensor 42 is shown in FIG. 26. The insertion gun 200 includes a housing 202 and a carrier 204. The insertion device 120 is typically mounted on the carrier 204 and the sensor 42 is pre-loaded into the insertion device 120. The carrier 204 drives the sensor 42 and, optionally, the insertion device 120 into the skin of the patient using, for example, a cocked or wound spring, a burst of compressed gas, an electromagnet repelled by a second magnet, or the like, within the insertion gun 200. In some instances, for example, when using a spring, the carrier 204 and insertion device may be moved, cocked, or otherwise prepared to be directed towards the skin of the patient.

After the sensor 42 is inserted, the insertion gun 200 may contain a mechanism which pulls the insertion device 120

US 6,565,509 B1

29

out of the skin of the patient. Such a mechanism may use a spring, electromagnet, or the like to remove the insertion device **120**.

The insertion gun may be reusable. The insertion device **120** is often disposable to avoid the possibility of contamination. Alternatively, the insertion device **120** may be sterilized and reused. In addition, the insertion device **120** and/or the sensor **42** may be coated with an anticlotting agent to prevent fouling of the sensor **42**.

In one embodiment, the sensor **42** is injected between 2 to 12 mm into the interstitial tissue of the patient for subcutaneous implantation. Preferably, the sensor is injected 3 to 9 mm, and more preferably 5 to 7 mm, into the interstitial tissue. Other embodiments of the invention, may include sensors implanted in other portions of the patient, including, for example, in an artery, vein, or organ. The depth of implantation varies depending on the desired implantation target.

Although the sensor **42** may be inserted anywhere in the body, it is often desirable that the insertion site be positioned so that the on-skin sensor control unit **44** can be concealed. In addition, it is often desirable that the insertion site be at a place on the body with a low density of nerve endings to reduce the pain to the patient. Examples of preferred sites for insertion of the sensor **42** and positioning of the on-skin sensor control unit **44** include the abdomen, thigh, leg, upper arm, and shoulder.

An insertion angle is measured from the plane of the skin (i.e., inserting the sensor perpendicular to the skin would be a 90° insertion angle). Insertion angles usually range from 10 to 90°, typically from 15 to 60°, and often from 30 to 45°.

On-skin Sensor Control Unit

The on-skin sensor control unit **44** is configured to be placed on the skin of a patient. The on-skin sensor control unit **44** is optionally formed in a shape that is comfortable to the patient and which may permit concealment, for example, under a patient's clothing. The thigh, leg, upper arm, shoulder, or abdomen are convenient parts of the patient's body for placement of the on-skin sensor control unit **44** to maintain concealment. However, the on-skin sensor control unit **44** may be positioned on other portions of the patient's body. One embodiment of the on-skin sensor control unit **44** has a thin, oval shape to enhance concealment, as illustrated in FIGS. 14-16. However, other shapes and sizes may be used.

The particular profile, as well as the height, width, length, weight, and volume of the on-skin sensor control unit **44** may vary and depends, at least in part, on the components and associated functions included in the on-skin sensor control unit **44**, as discussed below. For example, in some embodiments, the on-skin sensor control unit **44** has a height of 1.3 cm or less, and preferably 0.7 cm or less. In some embodiments, the on-skin sensor control unit **44** has a weight of 90 grams or less, preferably 45 grams or less, and more preferably 25 grams or less. In some embodiments, the on-skin sensor control unit **44** has a volume of about 15 cm³ or less, preferably about 10 cm³ or less, more preferably about 5 cm³ or less, and most preferably about 2.5 cm³ or less.

The on-skin sensor control unit **44** includes a housing **45**, as illustrated in FIGS. 14-16. The housing **45** is typically formed as a single integral unit that rests on the skin of the patient. The housing **45** typically contains most or all of the electronic components, described below, of the on-skin sensor control unit **44**. The on-skin sensor control unit **44** usually includes no additional cables or wires to other electronic components or other devices. If the housing

30

includes two or more parts, then those parts typically fit together to form a single integral unit.

The housing **45** of the on-skin sensor control unit **44**, illustrated in FIGS. 14-16, may be formed using a variety of materials, including, for example, plastic and polymeric materials, particularly rigid thermoplastics and engineering thermoplastics. Suitable materials include, for example, polyvinyl chloride, polyethylene, polypropylene, polystyrene, ABS polymers, and copolymers thereof. The housing **45** of the on-skin sensor control unit **44** may be formed using a variety of techniques including, for example, injection molding, compression molding, casting, and other molding methods. Hollow or recessed regions may be formed in the housing **45** of the on-skin sensor control unit **44**. The electronic components of the on-skin sensor control unit **44**, described below, and/or other items, such as a battery or a speaker for an audible alarm, may be placed in the hollow or recessed areas.

In some embodiments, conductive contacts **80** are provided on the exterior of the housing **45**. In other embodiments, the conductive contacts **80** are provided on the interior of the housing **45**, for example, within a hollow or recessed region.

In some embodiments, the electronic components and/or other items are incorporated into the housing **45** of the on-skin sensor control unit **44** as the plastic or polymeric material is molded or otherwise formed. In other embodiments, the electronic components and/or other items are incorporated into the housing **45** as the molded material is cooling or after the molded material has been reheated to make it pliable. Alternatively, the electronic components and/or other items may be secured to the housing **45** using fasteners, such as screws, nuts and bolts, nails, staples, rivets, and the like or adhesives, such as contact adhesives, pressure sensitive adhesives, glues, epoxies, adhesive resins, and the like. In some cases, the electronic components and/or other items are not affixed to the housing **45** at all.

In some embodiments, the housing **45** of the on-skin sensor control unit **44** is a single piece. The conductive contacts **80** may be formed on the exterior of the housing **45** or on the interior of the housing **45** provided there is a port **78** in the housing **45** through which the sensor **42** can be directed to access the conductive contacts **80**.

In other embodiments, the housing **45** of the on-skin sensor control unit **44** is formed in at least two separate portions that fit together to form the housing **45**, for example, a base **74** and a cover **76**, as illustrated in FIGS. 14-16. The two or more portions of the housing **45** may be entirely separate from each other. Alternatively, at least some of the two or more portions of the housing **45** may be connected together, for example, by a hinge, to facilitate the coupling of the portions to form the housing **45** of the on-skin sensor control unit **44**.

These two or more separate portions of the housing **45** of the on-skin sensor control unit **44** may have complementary, interlocking structures, such as, for example, interlocking ridges or a ridge on one component and a complementary groove on another component, so that the two or more separate components may be easily and/or firmly coupled together. This may be useful, particularly if the components are taken apart and fit together occasionally, for example, when a battery or sensor **42** is replaced. However, other fasteners may also be used to couple the two or more components together, including, for example, screws, nuts and bolts, nails, staples, rivets, or the like. In addition, adhesives, both permanent or temporary, may be used including, for example, contact adhesives, pressure sensitive adhesives, glues, epoxies, adhesive resins, and the like.

US 6,565,509 B1

31

Typically, the housing 45 is at least water resistant to prevent the flow of fluids into contact with the components in the housing, including, for example, the conductive contacts 80. Preferably, the housing is waterproof. In one embodiment, two or more components of the housing 45, for example, the base 74 and the cover 76, fit together tightly to form a hermetic, waterproof, or water resistant seal so that fluids can not flow into the interior of the on-skin sensor control unit 44. This may be useful to avoid corrosion currents and/or degradation of items within the on-skin sensor control unit 44, such as the conductive contacts, the battery, or the electronic components, particularly when the patient engages in such activities as showering, bathing, or swimming.

Water resistant, as used herein, means that there is no penetration of water through a water resistant seal or housing when immersed in water at a depth of one meter at sea level. Waterproof, as used herein, means that there is no penetration of water through the waterproof seal or housing when immersed in water at a depth of ten meters, and preferably fifty meters, at sea level. It is often desirable that the electronic circuitry, power supply (e.g., battery), and conductive contacts of the on-skin sensor control unit, as well as the contact pads of the sensor, are contained in a water resistant, and preferably, a waterproof, environment.

In addition to the portions of the housing 45, such as the base 74 and cover 76, there may be other individually-formed pieces of the on-skin sensor control unit 44, which may be assembled during or after manufacture. One example of an individually-formed piece is a cover for electronic components that fits a recess in the base 74 or cover 76. Another example is a cover for a battery provided in the base 74 or cover 76. These individually-formed pieces of the on-skin sensor control unit 44 may be permanently affixed, such as, for example, a cover for electronic components, or removably affixed, such as, for example, a removable cover for a battery, to the base 74, cover 76, or other component of the on-skin sensor control unit 44. Methods for affixing these individually-formed pieces include the use of fasteners, such as screws, nuts and bolts, staples, nails, rivets, and the like, frictional fasteners, such as tongue and groove structures, and adhesives, such as contact adhesives, pressure sensitive adhesives, glues, epoxies, adhesive resins, and the like.

One embodiment of the on-skin sensor control unit 44 is a disposable unit complete with a battery for operating the unit. There are no portions of the unit that the patient needs to open or remove, thereby reducing the size of the unit and simplifying its construction. The on-skin sensor control unit 44 optionally remains in a sleep mode prior to use to conserve the battery's power. The on-skin sensor control unit 44 detects that it is being used and activates itself. Detection of use may be through a number of mechanisms. These include, for example, detection of a change in resistance across the electrical contacts, actuation of a switch upon mating the on-skin sensor control unit 44 with a mounting unit 77 (see FIGS. 27A and 28A). The on-skin sensor control unit 44 is typically replaced when it no longer operates within threshold limits, for example, if the battery or other power source does not generate sufficient power. Often this embodiment of the on-skin sensor control unit 44 has conductive contacts 80 on the exterior of the housing 45. Once the sensor 42 is implanted in the patient, the sensor control unit 44 is placed over the sensor 42 with the conductive contacts 80 in contact with the contact pads 49 of the sensor 42.

The on-skin sensor control unit 44 is typically attached to the skin 75 of the patient, as illustrated in FIG. 17. The

32

on-skin sensor control unit 44 may be attached by a variety of techniques including, for example, by adhering the on-skin sensor control unit 44 directly to the skin 75 of the patient with an adhesive provided on at least a portion of the housing 45 of the on-skin sensor control unit 44 which contacts the skin 75 or by suturing the on-skin sensor control unit 44 to the skin 75 through suture openings (not shown) in the sensor control unit 44.

Another method of attaching the housing 45 of the on-skin sensor control unit 44 to the skin 75 includes using a mounting unit, 77. The mounting unit 77 is often a part of the on-skin sensor control unit 44. One example of a suitable mounting unit 77 is a double-sided adhesive strip, one side of which is adhered to a surface of the skin of the patient and the other side is adhered to the on-skin sensor control unit 44. In this embodiment, the mounting unit 77 may have an optional opening 79 which is large enough to allow insertion of the sensor 42 through the opening 79. Alternatively, the sensor may be inserted through a thin adhesive and into the skin.

A variety of adhesives may be used to adhere the on-skin sensor control unit 44 to the skin 75 of the patient, either directly or using the mounting unit 77, including, for example, pressure sensitive adhesives (PSA) or contact adhesives. Preferably, an adhesive is chosen which is not irritating to all or a majority of patients for at least the period of time that a particular sensor 42 is implanted in the patient. Alternatively, a second adhesive or other skin-protecting compound may be included with the mounting unit so that a patient, whose skin is irritated by the adhesive on the mounting unit 77, can cover his skin with the second adhesive or other skin-protecting compound and then place the mounting unit 77 over the second adhesive or other skin-protecting compound. This should substantially prevent the irritation of the skin of the patient because the adhesive on the mounting unit 77 is no longer in contact with the skin, but is instead in contact with the second adhesive or other skin-protecting compound.

When the sensor 42 is changed, the on-skin sensor control unit 44 may be moved to a different position on the skin 75 of the patient, for example, to avoid excessive irritation. Alternatively, the on-skin sensor control unit 44 may remain at the same place on the skin of the patient until it is determined that the unit 44 should be moved.

Another embodiment of a mounting unit 77 used in an on-skin sensor control unit 44 is illustrated in FIGS. 27A and 27B. The mounting unit 77 and a housing 45 of an on-skin sensor control unit 44 are mounted together in, for example, an interlocking manner, as shown in FIG. 27A. The mounting unit 77 is formed, for example, using plastic or polymer materials, including, for example, polyvinyl chloride, polyethylene, polypropylene, polystyrene, ABS polymers, and copolymers thereof. The mounting unit 77 may be formed using a variety of techniques including, for example, injection molding, compression molding, casting, and other molding methods.

The mounting unit 77 typically includes an adhesive on a bottom surface of the mounting unit 77 to adhere to the skin of the patient or the mounting unit 77 is used in conjunction with, for example, double-sided adhesive tape or the like. The mounting unit 77 typically includes an opening 79 through which the sensor 42 is inserted, as shown in FIG. 27B. The mounting unit 77 may also include a support structure 220 for holding the sensor 42 in place and against the conductive contacts 80 on the on-skin sensor control unit 44. The mounting unit 77, also, optionally, includes a positioning structure 222, such as an extension of material

US 6,565,509 B1

33

from the mounting unit 77, that corresponds to a structure (not shown), such as an opening, on the sensor 42 to facilitate proper positioning of the sensor 42, for example, by aligning the two complementary structures.

In another embodiment, a coupled mounting unit 77 and housing 45 of an on-skin sensor control unit 44 is provided on an adhesive patch 204 with an optional cover 206 to protect and/or confine the housing 45 of the on-skin sensor control unit 44, as illustrated in FIG. 28A. The optional cover may contain an adhesive or other mechanism for attachment to the housing 45 and/or mounting unit 77. The mounting unit 77 typically includes an opening 49 through which a sensor 42 is disposed, as shown in FIG. 28B. The opening 49 may optionally be configured to allow insertion of the sensor 42 through the opening 49 using an insertion device 120 or insertion gun 200 (see FIG. 26). The housing 45 of the on-skin sensor control unit 44 has a base 74 and a cover 76, as illustrated in FIG. 28C. A bottom view of the housing 45, as shown in FIG. 28D, illustrates ports 230 through which conductive contacts (not shown) extend to connect with contact pads on the sensor 42. A board 232 for attachment of circuit components may optionally be provided within the on-skin sensor control unit 44, as illustrated in FIG. 28E.

In some embodiments, the adhesive on the on-skin sensor control unit 44 and/or on any of the embodiments of the mounting unit 77 is water resistant or waterproof to permit activities such as showering and/or bathing while maintaining adherence of the on-skin sensor control unit 44 to the skin 75 of the patient and, at least in some embodiments, preventing water from penetrating into the sensor control unit 44. The use of a water resistant or waterproof adhesive combined with a water resistant or waterproof housing 45 protects the components in the sensor control unit 44 and the contact between the conductive contacts 80 and the sensor 42 from damage or corrosion. An example of a non-irritating adhesive that repels water is Tegaderm (3M, St. Paul, Minn.).

In one embodiment, the on-skin sensor control unit 44 includes a sensor port 78 through which the sensor 42 enters the subcutaneous tissue of the patient, as shown in FIGS. 14 to 16. The sensor 42 may be inserted into the subcutaneous tissue of the patient through the sensor port 78. The on-skin sensor control unit 44 may then be placed on the skin of the patient with the sensor 42 being threaded through the sensor port 78. If the housing 45 of the sensor 42 has, for example, a base 74 and a cover 76, then the cover 76 may be removed to allow the patient to guide the sensor 42 into the proper position for contact with the conductive contacts 80.

Alternatively, if the conductive contacts 80 are within the housing 45 the patient may slide the sensor 42 into the housing 45 until contact is made between the contact pads 49 and the conductive contacts 80. The sensor control unit 44 may have a structure which obstructs the sliding of the sensor 42 further into the housing once the sensor 42 is properly positioned with the contact pads 49 in contact with the conductive contacts 80.

In other embodiments, the conductive contacts 80 are on the exterior of the housing 45 (see e.g., FIGS. 27A–27B and 28A–28E). In these embodiments, the patient guides the contact pads 49 of the sensor 42 into contact with the conductive contacts 80. In some cases, a guiding structure may be provided on the housing 45 which guides the sensor 42 into the proper position. An example of such a structure includes a set of guiding rails extending from the housing 45 and having the shape of the sensor 42.

In some embodiments, when the sensor 42 is inserted using an insertion device 120 (see FIG. 12), the tip of the

34

insertion device 120 or optional insertion gun 200 (see FIG. 26) is positioned against the skin or the mounting unit 77 at the desired insertion point. In some embodiments, the insertion device 120 is positioned on the skin without any guide. In other embodiments, the insertion device 120 or insertion gun 200 is positioned using guides (not shown) in the mounting unit 77 or other portion of the on-skin sensor control unit 44. In some embodiments, the guides, opening 79 in the mounting unit 77 and/or sensor port 78 in the housing 45 of the on-skin sensor control unit 44 have a shape which is complementary to the shape of the tip of the insertion device 120 and/or insertion gun 200 to limit the orientation of the insertion device 120 and/or insertion gun 200 relative to the opening 79 and/or sensor port 78. The sensor can then be subcutaneously inserted into the patient by matching the complementary shape of the opening 79 or sensor port 78 with the insertion device 120 and/or insertion gun 200.

In some embodiments, the shapes of a) the guides, opening 79, or sensor port 78, and (b) the insertion device 120 or insertion gun 200 are configured such that the two shapes can only be matched in a single orientation. This aids in inserting the sensor 42 in the same orientation each time a new sensor is inserted into the patient. This uniformity in insertion orientation may be required in some embodiments to ensure that the contact pads 49 on the sensor 42 are correctly aligned with appropriate conductive contacts 80 on the on-skin sensor control unit 44. In addition, the use of the insertion gun, as described above, may ensure that the sensor 42 is inserted at a uniform, reproducible depth.

The sensor 42 and the electronic components within the on-skin sensor control unit 44 are coupled via conductive contacts 80, as shown in FIGS. 14–16. The one or more working electrodes 58, counter electrode 60 (or counter/reference electrode), optional reference electrode 62, and optional temperature probe 66 are attached to individual conductive contacts 80. In the illustrated embodiment of FIGS. 14–16, the conductive contacts 80 are provided on the interior of the on-skin sensor control unit 44. Other embodiments of the on-skin sensor control unit 44 have the conductive contacts disposed on the exterior of the housing 45. The placement of the conductive contacts 80 is such that they are in contact with the contact pads 49 on the sensor 42 when the sensor 42 is properly positioned within the on-skin sensor control unit 44.

In the illustrated embodiment of FIGS. 14–16, the base 74 and cover 76 of the on-skin sensor control unit 44 are formed such that, when the sensor 42 is within the on-skin sensor control unit 44 and the base 74 and cover 76 are fitted together, the sensor 42 is bent. In this manner, the contact pads 49 on the sensor 42 are brought into contact with the conductive contacts 80 of the on-skin sensor control unit 44. The on-skin sensor control unit 44 may optionally contain a support structure 82 to hold, support, and/or guide the sensor 42 into the correct position.

Non-limiting examples of suitable conductive contacts 80 are illustrated in FIGS. 19A–19D. In one embodiment, the conductive contacts 80 are pins 84 or the like, as illustrated in FIG. 19A, which are brought into contact with the contact pads 49 on the sensor 42 when the components of the on-skin sensor control unit 44, for example, the base 74 and cover 76, are fitted together. A support 82 may be provided under the sensor 42 to promote adequate contact between the contact pads 49 on the sensor 42 and the pins 84. The pins are typically made using a conductive material, such as a metal or alloy, for example, copper, stainless steel, or silver. Each pin has a distal end that extends from the on-skin